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ORIGINAL ARTICLE

Decreases in Mineralocorticoid but not Glucocorticoid Receptor mRNA Expression During the Short Arctic Breeding Season in Free-Living Gambel's White-Crowned Sparrow (*Zonotrichia leucophrys gambelii*)

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The acute stress response in vertebrates is a highly adaptive suite of physiological and behavioural mechanisms that promote survival in the face of deleterious stimuli from the environment. Facultative changes of physiology and behaviour are mediated through changes in circulating levels of glucocorticoids (corticosterone, cortisol) and their subsequent binding to the high-affinity mineralocorticoid receptor (MR) or the low-affinity glucocorticoid receptor (GR). Free-living male wild Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*) display annual fluctuations in the stress response with marked attenuation during the transition from the pre-parental to the parental stage. We investigated whether this rapid reduction in the stress response is mediated through changes in MR and GR mRNA expression in the brain using *in situ* hybridisation. MR mRNA expression was found to be significantly lower in the hippocampus as the male birds became parental. No changes were observed in GR mRNA expression in the paraventricular nucleus (PVN) or preoptic area (POA) at this time. No significant correlations were found between initial capture levels of corticosterone and GR or MR mRNA expression. No differences were found in basal levels of corticosterone between pre-parental and parental in birds collected for *in situ* hybridisation. Stress response data revealed no difference at baseline but reductions in peak levels of corticosterone as birds became parental. These data suggest that changes in MR expression may be important for the regulation of the stress response or behavioural stress sensitivity with respect to promoting parental care and investment.

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Every spring, migratory songbirds depart from their wintering grounds and fly thousands of kilometres to their breeding grounds where they may encounter demanding conditions, such as intense storms, food shortages, predators and social disputes (1). During such times, songbirds are reliant upon the hypothalamic-pituitary-adrenal (HPA) axis and the production of the stress hormone, corticosterone, to regulate the behavioural and physiological changes that promote their survival. On the acute time scale, the activity of this system is highly adaptive and promotes survival by allocating resources away from unnecessary physiological processes towards those that are essential for self-preservation (1–3). A possible constraint is that chronic activity of the HPA axis can have deleterious effects on the organism, such as decreases in immune function, sexual behaviour, reproductive success and parental care (3).

The stress response is highly plastic and changes as an animal progress through life-history stages (e.g. for birds breeding, plumage moult, migration and nonbreeding) to meet the differing seasonal and energetic demands of the annual cycle (4). Seasonal changes in stress physiology have been described in all tetrapod vertebrates (5) and corticosterone secretion in response to acute restraint stress varies widely by season in wild Arctic breeding migratory songbirds. Specifically, basal and stressed-induced levels are highest during the onset of breeding (6–9). This elevation in HPA axis activity promotes a more rapid and intense response to perturbations, which not only quickly directs energy toward survival enhancing activities, but also allows the individual to return to breeding sooner, which enhances fitness (10). As birds transition from the pre-parental to the parental stage of breeding, HPA axis

activity is down-regulated to promote parental investment (9,11,12). Parents with an active nest have invested a substantial amount of energy and reduce HPA axis activity to ensure the brood is raised to fledging (12). However, in Arctic-breeding songbirds, nest abandonment is promoted during intense prolonged snow storms as a result of chronically elevated levels of corticosterone (13).

The stress response is a complex system with tissue specific effects that are orchestrated through multiple levels of regulation, including circulating levels of hormone and corticosteroid binding-globulin (CBG), genomic and membrane bound receptor expressions, and 11 β -hydroxysteroid dehydrogenase (11 β -HSD1 and 2) activity (14). CBG fluctuates in parallel with the annual changes in the stress response to regulate corticosterone availability at the plasma and cellular level so the free fraction of corticosterone remains constant (15). 11 β -HSD2 is an enzyme that converts glucocorticoids into the less active metabolites and 11 β -HSD1 performs the reverse reaction, although little is known about its seasonal expression (16). The expression of both isoforms of 11 β -HSD across the year could greatly affect the degree of receptor saturation by controlling steroid availability. Ultimately, stress hormone receptor expression is the most critical component of this system because all other points of modulation simply regulate the amount of hormone that may interact with the receptor. Elevation of corticosterone during stress leads to receptor saturation that reduces HPA activity through negative-feedback.

In birds and mammals, there are two intracellular genomic receptors that bind to corticosterone and less well studied membrane bound receptors (17). Both genomic receptors are transcriptional factors with a zinc finger motif and bind to their respective glucocorticoid responsive elements to influence gene transcription (18). Membrane bound receptors interact with second messenger systems to promote rapid changes in cell activity (19). The genomic mineralocorticoid receptor (MR, type I) has a 10-fold higher binding affinity for corticosterone than the genomic glucocorticoid receptor (GR, type II). This difference in binding affinity is assumed to create a two-tier system for responding to basal and stress-induced levels of corticosterone with negative-feedback mediated by receptors according to ligand affinity (20). Basal corticosterone levels feedback on high-affinity genomic MR, whereas peak induced levels feedback on low-affinity genomic GR and membrane bound MR. In mammals, negative-feedback on HPA activity has been linked to the hippocampus, paraventricular nucleus (PVN) in the hypothalamus and the pituitary gland, although regulatory mechanisms remain poorly understood in birds. Lesion and receptor antagonist studies in mammals and birds suggest that MR in the hippocampus has an inhibitory influence on basal HPA activity, likely through inputs to the PVN (21–23). GR regulates negative-feedback to terminate the stress response in rats because the administration of RU486, a selective GR antagonist, leads to elevations in circadian peaks and stress-induced levels of corticosterone (24). Studies on rats indicate that the stress response is reduced at the end of pregnancy and through early lactation with associated changes in GR in the PVN but no changes in MR expression (25). Chronic stress in European starlings (*Sturnus vulgaris*) reduces basal and peak corticosterone

levels (26) and these changes are likely mediated through reductions in expression of MR in the hippocampus and GR in the PVN (27).

MR and GR expression in key brain regions is important for modifying the behavioural responses to a variety of stimuli. Transgenic mice that over-express MR show marked increases in spatial memory and reduced anxiety-related behaviours (28). In birds, the transition from the pre-parental to parental stage of breeding brings with it a suite of behavioural changes that are necessary for the proper care and provisioning of chicks. This transition may be mediated via the differential expression of MR and GR receptors in the brain. In Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*), corticosterone elevation, by use of s.c. implants, has been shown to decrease aggression, at the same time as increasing both the foraging rate and locomotion (8,29,30).

The present study aimed to investigate the regulatory negative-feedback mechanism responsible for the reduction in the stress response during the transition to the parental phase in free-living male Gambel's white-crowned sparrows by quantifying the changes in MR and GR mRNA expression. To our knowledge, this is the first study to investigate changes in stress hormone receptor expression with the aim of understanding stress response plasticity in a wild population of Arctic songbirds. We tested our hypothesis proposing that changes in the stress response during the transition from the pre-parental to parental phase are mediated by changes in stress hormone receptor expression. We report stress response data from individuals captured at the two stages of breeding.

Materials and methods

Birds

Gambel's white-crowned sparrow is one of five subspecies of *Z. leucophrys*. This subspecies is the longest distance migrant wintering in the American southwest and breeding in northern Canada and Alaska (31). The preferred habitat is dominated by low stature shrubs (32). These birds are considered to be socially monogamous and raise a single clutch during the short breeding season. Males do not incubate eggs, or feed the females when they are on the nest, although they feed nestlings and fledglings (31).

In situ hybridisation for MR and GR was performed on sixteen males that were collected during the 2011 breeding season in the vicinity of Toolik Lake Field Station located on the North Slope of Alaska (69°38' N, 149°36' W). All brains were collected between 10.00 h and 16.00 h. Circadian peaks in corticosterone occur prior to sunrise or approximately 01.00 h for captive birds housed on under 20 hours of light and 4 h of darkness (33). The first eight males (pre-parental) were collected on 5 and 7 June and, at that time, females were incubating eggs. Another eight males (parental) were collected on 18 June at the onset of nestling feeding. A standardised restraint handling protocol was used to assess corticosterone secretion at basal and stress-induced levels (34) in four males during the pre-parental phase and three during the parental phase. Stress series sampling was conducted within 3 days of euthanasia sampling. At the time of capture, morphometric measurements of tarsus and wing length, fat score on scale from 0–5 (35) and body weight were collected from each bird. An initial blood sample was collected from the alar vein within 3 min of capture for corticosterone quantification in birds collected for *in situ* hybridisation. Birds were sedated in the field with isoflurane and decapitated with a mean (\pm SEM) time from

capture to euthanasia of 3 min and 45 s (\pm 20 s). The brain was immediately frozen on dry ice and stored at -80°C . All work was approved by University of California Davis Institutional Animal Care and Use Committee under protocol 16283.

Radioimmunoassay for corticosterone

A radioimmunoassay for corticosterone was used to quantify hormone levels (34). Briefly, plasma volumes were measured (30 μl) and equilibrated with 2000 c.p.m. of tritiated corticosterone to determine recoveries. Steroids were extracted, for approximately 3 h, using freshly redistilled dichloromethane, dried under nitrogen at 35°C , and reconstituted in 550 μl of phosphate-buffered saline with gelatine. The next day, 200 μl was placed in duplicate assay tubes, and 100 μl was used to determine the percent recovery. Each assay tube received 100 μl (approximately 10 000 c.p.m.) of tritiated corticosterone (Perkin Elmer, Boston, MA, USA) and 100 μl of antiserum (B3-163; Esoterix Inc., Austin, TX, USA). Unbound steroid was stripped using 500 μl of dextran-coated charcoal followed by centrifugation. The supernatant was decanted, combined with scintillation fluid (Ultima Gold; Perkin Elmer), and then counted for 10 min or within 2% accuracy on a Beckman 6500 scintillation counter (Beckman Coulter, Fullerton, CA, USA). Mean recovery for corticosterone was 90%. The mean detection limit for the assay was 0.8 ng/ml. All basal samples for the hybridisation study were run within the same assay with an intra-assay variation of 10.28%. Stress series samples were run across multiple assays with an inter- and intra-assay variation of 8.94 and 9.07, respectively.

In situ hybridisation histochemistry for MR and GR mRNA

Whole brains were sectioned coronally at 15 μm on a cryostat and thaw mounted onto polysine, RNAase free, pre-treated glass microscope slides. Marker slides were created by collecting every sixth section and then stained using a cresyl violet stain. Sections were stored at -80°C with silica pellets until the *in situ* hybridisation was performed. Slides for hybridisation work were selected after examination of marker slides in conjunction with the canary stereotaxic atlas (36) to locate regions of interest.

The MR and GR hybridisation procedures have been described in detail previously (27,37). Briefly, 500-bp fragments of the zebra finch GR (Genbank: XM_002186722) or MR (Genbank: DQ539433) were subcloned into PGEM-7. GR sense and antisense riboprobes were generated by *in vitro* transcription, in the presence of 35S-UTP, with SP6- and T7-RNA polymerase after plasmid linearisation with *EcoRI* or *HindIII*, respectively. MR sense and antisense riboprobes were generated by *in vitro* transcription, in the presence of 35S-UTP, with T7- and SP6-RNA polymerase after plasmid linearisation with *HindIII* or *ApoI*, respectively. The clones were generously provided by Drs M. Gahr and R. Metzdorf (Department of Behavioural Neurobiology Max-Planck-Institute for Ornithology, Seewiesen, Germany). Both GR and MR are highly conserved genes, with identities between zebra finch (passerine) and chicken (galliform) of 88% and 90%, respectively (27).

Slides were dipped in autoradiography emulsion to visualise the hybridised cells and exposed for 6 weeks. Autoradiographs were then counter-stained with haematoxylin and eosin and cover-slipped with DPX mountant (Sigma, St Louis, MO, USA). Hybridisation of sections with GR or MR sense riboprobes, or pre-treatment with RNase-A prior to hybridisation with the GR or MR antisense riboprobes, did not result in any detectable hybridisation signal.

Image analysis

Slides were examined under bright field microscopy using a Zeiss Axioskop 40 (Carl Zeiss Meditec, Dublin, CA, USA) and images were captured using an axiocam MRc5 camera (Carl Zeiss Meditec Inc.). Images were analysed using

IMAGEJ (NIH, Bethesda, MD, USA). Anatomical structures were determined in combination with the canary stereotaxic atlas (36) and marker slides to locate brain regions containing the POA, PVN, hippocampus and nucleus septalis medialis. Slides were coded so that the observer was blind to the two groups.

Quantification of MR and GR mRNA expression

Cells counts

The number of cells hybridised for MR or GR mRNA were counted only when silver grain labelling exceeded three times background for an equivalent labelled region. In the PVN, hippocampus, POA and nucleus septalis medialis, all of the hybridised cells were counted in the defined anatomical region of interest. Counts were conducted for each of the four sections per slide and each bird had two slides. Mean hybridised cells were then calculated for each individual per hemisphere for the region of interest.

Optical density

Signal density was measured using IMAGEJ. We quantified silver grain density, which appears black under bright field microscopy, in the autoradiograph by first converting the image to 8-bit colour, inverted the colours so that hybridised cells appeared white, and then took an optical density measurement. In the PVN, we chose to outline the region of cells expressing hybridisation as opposed to using boxes with fixed areas. A background measurement was taken from an area adjacent to the PVN. For the hippocampus, we used two methods of analyses. In the first method, a standardised box was randomly placed in five different locations within the hippocampus and measurements were recorded. In the second method, we outlined the entire hippocampus and took one measurement. By outlining the entire hippocampus, we were able to eliminate having to take five repeated measurements as described in the first method. A background measurement for the hippocampus was taken along the midline of the two hemispheres where hybridisation was not present.

Statistical analysis

Statistical analyses were performed using JMP, version 7 (SAS Institute Inc., Cary, NC, USA). All data were tested for deviations from normality using Shapiro-Wilks test ($P < 0.05$) and homogeneity of variance using Levene's test ($P < 0.05$) and were found to meet the assumptions for parametric tests. To evaluate effect of parental stage on body mass, two separate analyses were performed using a Student's two-tailed t-test. First, we compared mass directly between the two stages. Second, we corrected for body size by using a principle component analysis using wing chord and tarsus to create a body size index (PC1). The residuals from the regression of body mass against the PC1 were used to create a corrected body condition index measure that was then used to compare groups. A composite fat score was generated, by summing furcular and abdominal fat scores, and the ordinal data were analysed using a chi-squared test to investigate differences between stages. Cell counts and optical density measurements were analysed using a Student's two-tailed t-test to determine significant differences between mean values for each group. Stress series data were analysed using a repeated measures ANOVA with the main effect of breeding stage. Post-hoc analysis was performed using a Student's two-tailed t-test to determine differences for basal and peak induced levels of corticosterone.

Results

Circulating levels of corticosterone

Circulating levels of basal corticosterone at capture in the birds used for the *in situ* hybridisation study did not differ between pre-parental (4.41 ± 1.2 ng/ml) and parental (4.41 ± 1.3 ng/ml) individuals ($t = 0.23$, $P = 0.85$). Corticosterone levels increased significantly in response to a standardised restraint handling paradigm in birds captured to measure the responsiveness of the HPA axis to acute stress ($F_{1,10} = 159.23$, $P < 0.001$). There was a significant interaction of stress and stage on circulating levels of corticosterone ($F_{1,10} = 13.38$, $P = 0.004$). Basal levels of corticosterone were not different between pre-parental and parental stages ($t = 1.26$, $P = 0.22$). Stress-induced levels were significantly higher during the pre-parental stage ($t = 3.33$, $P = 0.007$) (Fig. 1). There was no significant correlation between basal circulating levels of corticosterone and the average number of cells hybridised for GR ($r = 0.15$, $P = 0.59$) or MR ($r = 0.01$, $P = 0.94$).

Distribution of MR and GR mRNA expression

Glucocorticoid receptor was not widely expressed throughout the brain and was limited to the PVN and POA in the diencephalon and the high vocal centre (HVC) and nidopallium in the telencephalon (Fig. 2). GR expression in the hippocampus was present but expression was too low to be quantified.

Mineralocorticoid receptor had a greater distribution throughout the telencephalon, with expression occurring in the hippocampus, HVC, nidopallium, nucleus rotundus and nucleus septalis medialis. MR hybridisation in the hippocampus (Fig. 2) was found within the 'V' arms, as previously described by Dickens *et al.* (38); this region

of the hippocampus was suggested to be analogous to the mammalian dentate gyrus of the hippocampus.

GR mRNA expression in the paraventricular nucleus

There was no significant difference in the number of cells hybridised ($t = 0.39$, $P = 0.74$) or the optical density in the PVN ($t = -1.22$, $P = 0.24$) (Fig. 3).

MR mRNA expression in the hippocampus

Transition from the pre-parental to parental stage resulted in a 21% decrease in the number of cells hybridised for the MR receptor in the hippocampus ($t = 2.67$, $P = 0.02$). There was also a 22% decrease in optical density in parental birds when analysed using five repeated measurements from the hippocampus ($t = 2.47$, $P = 0.02$). When the entirety of the hippocampus was outlined and a single optical density measurement was taken, the results indicated a 32% reduction in MR density in parental birds ($t = 2.42$, $P = 0.03$) (Fig. 4).

GR mRNA expression in the preoptic area

Glucocorticoid receptor mRNA cell counts ($t = -0.19$, $P = 0.84$) or optical density ($t = -0.77$, $P = 0.44$) did not significantly differ between the pre-parental and parental stages of breeding in the POA (Fig. 5).

MR mRNA receptor expression in the nucleus septalis medialis

No significant differences were found in the number of hybridised cells ($t = 0.60$, $P = 0.55$) or optical density ($t = -0.66$, $P = 0.51$) in the nucleus septalis medialis (Fig. 6).

Body morphometrics

Fat levels were not different between the pre-parental (3.3 ± 0.26) and parental (3.42 ± 0.36) stages of breeding ($\chi^2 = 4.53$, $P = 0.10$, data not shown). Mass was significantly lower during the pre-parental (24.31 ± 0.51 g) compared to the parental (25.90 ± 0.29 g) phase ($t = -2.72$, $P = 0.01$, data not shown). When corrected for body size, body condition index also indicated lower mass during the pre-parental stage ($t = -2.19$, $P = 0.04$).

Discussion

Free-living male Gambel's white-crowned sparrows transitioning from the pre-parental to the parental stage of breeding showed a significant decline in hippocampal MR mRNA expression, whereas GR mRNA expression remained unchanged. The results from the present study, as well as those from previous studies, using free-living male Gambel's white-crowned sparrows demonstrate the plasticity of the stress response with a seasonal peak in corticosterone in response to acute restraint being coincident with the pre-parental stage and then declining as birds transition to the parental stage (11). Below, we discuss three potential explanations for the

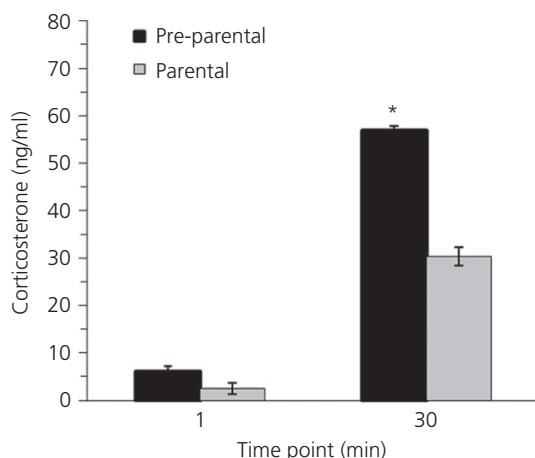


Fig. 1. The effect of standardised restraint handling on circulating levels of corticosterone during the pre-parental and parental stages of breeding in male Gambel's white-crowned sparrows. No significant differences were found at the basal level but stress-induced levels differed significantly by stage of breeding. All values are presented as the mean \pm SEM. * $P < 0.05$.

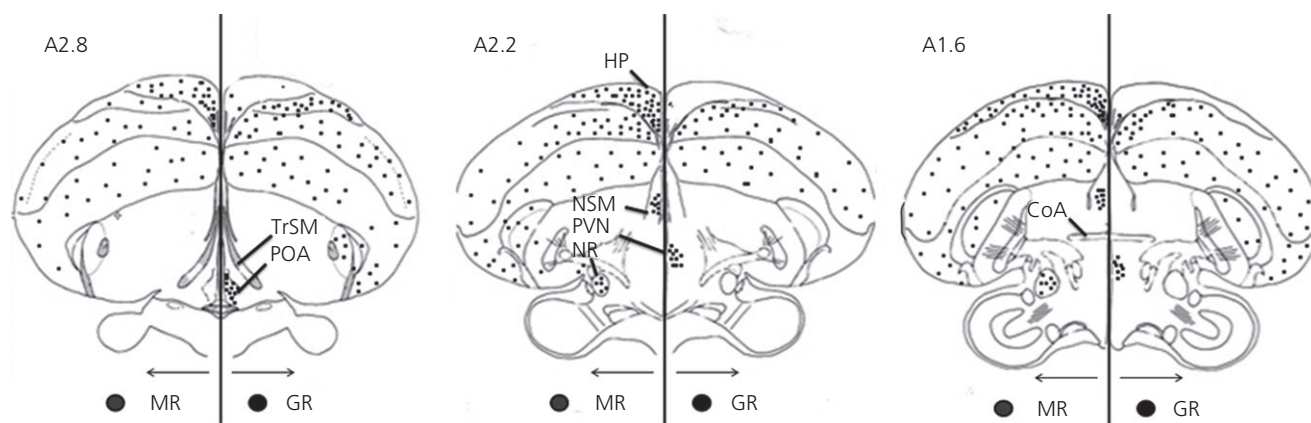


Fig. 2. Schematic of coronal brain sections summarising the distribution of mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) mRNA expression. The approximate anatomical location of each section is determined by Stokes *et al.* (1974), as indicated at the top left of each section. Each coronal section is divided down the midline, with MR expression on the left and GR on the right. Approximate expression is indicated by filled circles for each receptor. TrSM, tractus septomesencephalicus; POA, preoptic area; HP, hippocampus; NSM, nucleus septalis medialis; PVN, paraventricular nucleus; NR, nucleus rotundus; CoA, commissura anterior.

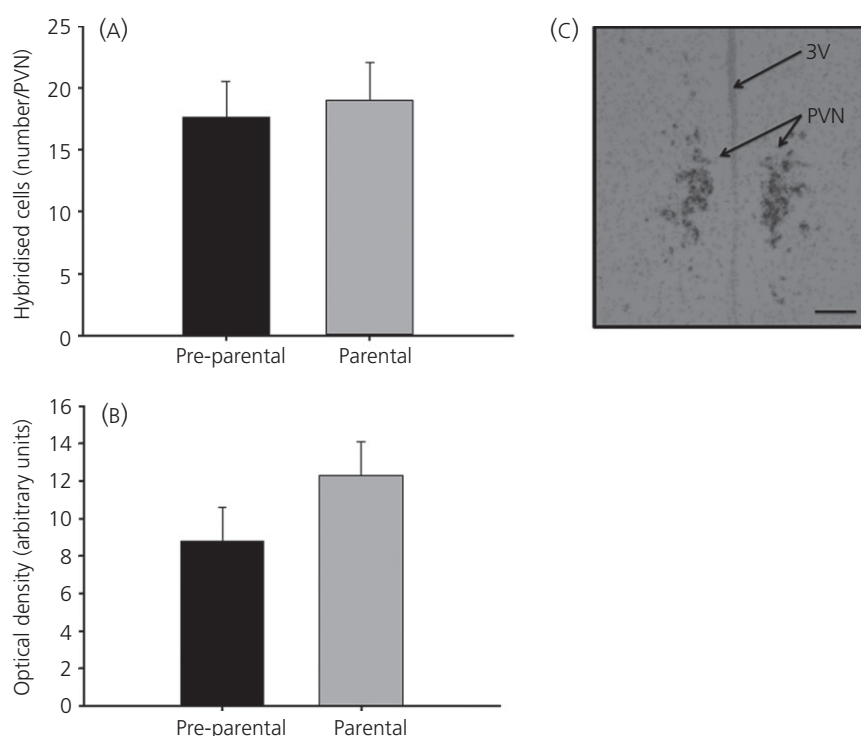


Fig. 3. Expression of glucocorticoid (GR) receptor mRNA in pre-parental ($n = 8$) and parental ($n = 8$) male Gambel's white-crowned sparrows in the paraventricular nucleus (PVN). GR expression is presented as (A) the mean number of cells hybridised per PVN and (B) the mean optical density per PVN. There was no statistical difference between the pre-parental and parental stages for three methods of analyses ($P > 0.05$). (C) A representative photomicrograph of autoradiograph in bright field of GR mRNA expressed in the PVN. The image is centred on the third ventricle (3V). Scale bar = 50 μm . All values are presented as the mean \pm SEM.

change in MR based on our current understanding of genomic receptors, membrane bound receptors and the ratio of MR : GR. In addition, we discuss our findings for GR expression in the hippocampus and PVN.

HPA activity is regulated by corticotrophin-releasing hormone (CRH) and arginine vasotocin (AVT) neurones residing within the PVN that integrate information from the limbic system and brain stem, as well as negative-feedback through circulating levels of

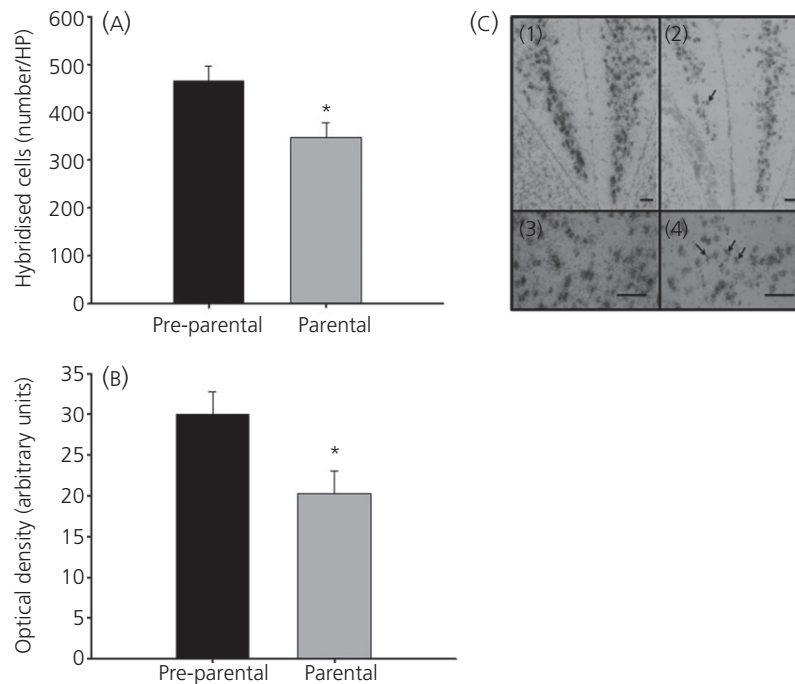


Fig. 4. Expression of mineralocorticoid (MR) receptor mRNA in pre-parental ($n = 8$) and parental ($n = 8$) male Gambel's white-crowned sparrows in the hippocampus. MR expression is presented as (A) the mean number of cells hybridised and (B) the mean optical density per hemisphere of the hippocampus. HP, hippocampus. All values are presented as the mean \pm SEM. * $P < 0.05$. Representative photomicrographs of autoradiographs in bright field showing cells expressing mineralocorticoid receptor (MR) mRNA in hippocampus (C) from (1) and (3) pre-parental and (2) and (4) parental Gambel's white-crowned sparrows. Arrows demonstrate examples of cells expressing MR mRNA (hybridised with MR antisense riboprobe *in situ* hybridisation visible as silver grains over cell bodies). Scale bars = 50 μ m.

glucocorticoids. The hippocampus is part of the limbic system and has been suggested to play an important role in regulating basal and, to a certain extent, stress-induced levels of corticosterone through negative-feedback on genomic MR and GR. In the present study, birds during the pre-parental stage showed elevated MR mRNA expression. Experiments conducted in rats indicate that treatment with progesterone increases MR mRNA expression in hippocampal cells (39). Progesterone can be detected in the blood of male Gambel's white-crowned sparrows, although seasonal variation was absent, which indicated that the source is probably primarily extra-gonadal (40). Because progesterone likely did not influence expression, these data based on the mammalian literature suggest that a decrease in HPA axis sensitivity to negative-feedback should result in an elevation in circulating levels of corticosterone both at baseline and under stress (41,42). However, we failed to detect a difference in baseline corticosterone levels in the birds collected for *in situ* hybridisation, whereas stress response data indicated an attenuation of corticosterone secretion in response to capture restraint handling. Genomic MR in Gambel's white-crowned sparrows are more likely to control baseline and circadian changes in corticosterone, although HPA axis activation in response to acute stress may act through nongenomic pathways to control stress-induced levels of corticosterone to facilitate rapid physiological and behavioural responses (43).

Feedback inhibition on the HPA axis is integrated by both genomic and membrane bound MR. Genomic MR can be shuttled from

the nucleus or cytoplasm into the membrane to act as a membrane bound receptor that rapidly affects neuronal activity (19). If the membrane bound receptor is a genomic receptor that has migrated to the membrane, then *in situ* hybridisation for mRNA will not distinguish between the two potential site locations and presents a potential confound when interpreting the results. Positioning the MR in the membrane results in a 10-fold reduction in the affinity for corticosterone and only allows for activation during periods of elevated corticosterone levels (19). In rats, blockade of the membrane bound receptor leads to a rapid increase in corticosterone levels by decreasing the inhibitory tone on the PVN (44). It is currently assumed that the membrane bound MR facilitates the stress response by synergising with other signalling molecules such as CRH, AVT and adrenaline to increase neuronal activity in the hippocampus, which promotes vigilance, appraisal and emotional reactivity during the stressor (45). In addition, elevated expression of MR mRNA imbues neural protection from cell apoptosis in rats (28) and may provide a protective mechanism in Gambel's white-crowned sparrows during the pre-parental stage when HPA axis activity is enhanced. A reduction in MR mRNA expression at the parental stage could decrease hippocampal neuronal activation and thereby reduce emotional reactivity during the stress response. Behavioural responses to corticosterone administration to Gambel's white-crowned sparrow have been shown to promote locomotor activity, be permissive for foraging, and decrease aggressive responses to standardised territorial intrusions (8,29,46). These findings would

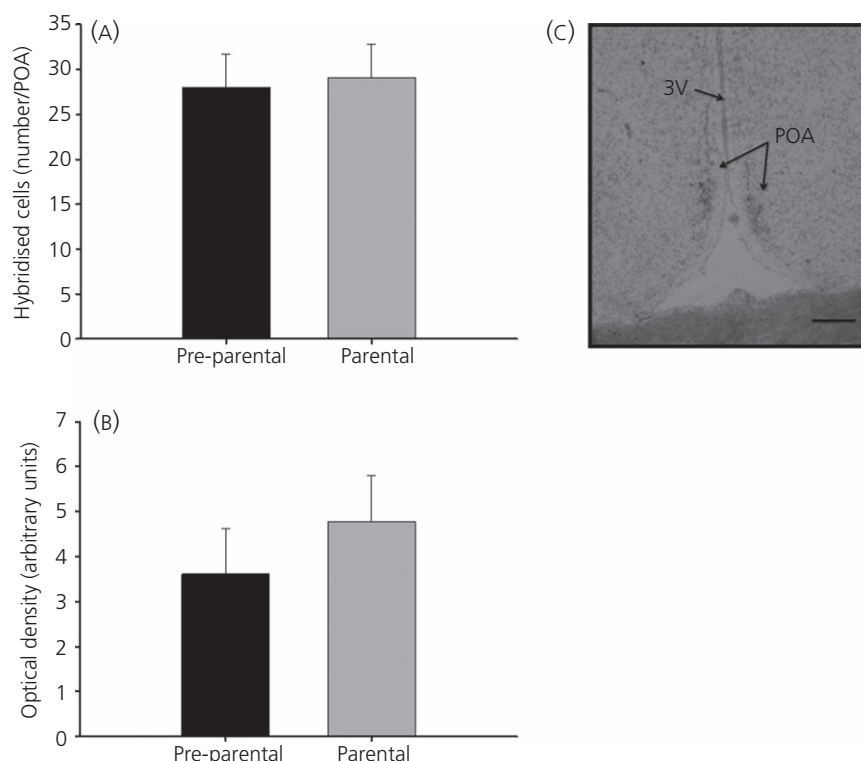


Fig. 5. Expression of glucocorticoid (GR) receptor mRNA in pre-parental ($n = 8$) and parental ($n = 8$) male Gambel's white-crowned sparrows in the preoptic area (POA). GR mRNA expression is presented as (A) the mean number of cells hybridised and (B) the mean optical density per hemisphere of the hippocampus. There is no significant difference between groups ($P > 0.05$). All values are presented as the mean \pm SEM. A representative photomicrograph is shown in (C). 3V, third ventricle. Scale bar = 200 μ m.

suggest that the changes in MR expression may control behavioural responses to a stressor. Membrane bound receptors could modulate the responsiveness to stress or affect the priming process for long-term effects mediated by genomic receptors.

There were no significant differences in the number of cells expressing MR mRNA in the nucleus septalis medialis. The nucleus septalis medialis sends afferent signals to the hippocampus and promotes the release of acetylcholine (47). The nucleus septalis medialis is considered to play a role in reward and reinforcement along with the nucleus accumbens, as well as the regulation of circadian patterns of corticosterone secretion by controlling inhibitory tone in the hypothalamus (48).

Classically, GR expression has been suggested to control peak corticosterone levels via a negative-feedback mechanism during acute stress. GR mRNA expression within the brain was similar to that previously reported for the European starling (*Sturnus vulgaris*), Zebra finch (*Taeniopygia guttata*) and Chukar (*Alectoris chukar*) (27,37,49). GR mRNA expression in the PVN or POA was not different between the pre-parental and parental stages. Corticosterone feeds back via GR both in the PVN and pituitary to terminate the stress response. In Gambel's white-crowned sparrows, the administration of dexamethasone suppresses stress-induced elevation of corticosterone (50), suggesting that negative-feedback occurs at the level of the pituitary gland. Changes in receptor density or cells expressing GR mRNA in the PVN would have suggested a change in

feedback sensitivity; however, we did not find such a difference in the present study. In rodent studies, a reduction in the stress response as females approach the end of pregnancy and initiate lactation is mediated through reductions in GR expression in the PVN along with changes in 11 β -HSD expression (25). We cannot dismiss the possibility that alterations in the stress response are not the result of other centres in the brain such as the extra-hypothalamic GABAergic neurones or the very perception of the stressor itself (51).

In rats, hippocampal GR also controls basal levels of corticosterone during the circadian peak and in response to stress (20). GR expression in the hippocampus in the present study was very limited and was unquantifiable, both in the pre-parental and parental stages. GR blockade, using the selective antagonist RU486, leads to decreases in basal levels of corticosterone in Gambel's white-crowned sparrows in winter condition or displaying nocturnal restlessness (46). Inhibition of the GR would indicate a role in the regulation of baseline corticosterone and these results are counter to what is typically seen in rodents after the administration of a selective GR antagonist during the circadian nadir. Baseline levels of corticosterone are regulated by input from the hippocampus, nucleus septalis medialis and nucleus medialis posterioris hypothalamus (22). Gambel's white-crowned sparrows exhibit diurnal activity, with peak corticosterone levels occurring several hours before dawn (33).

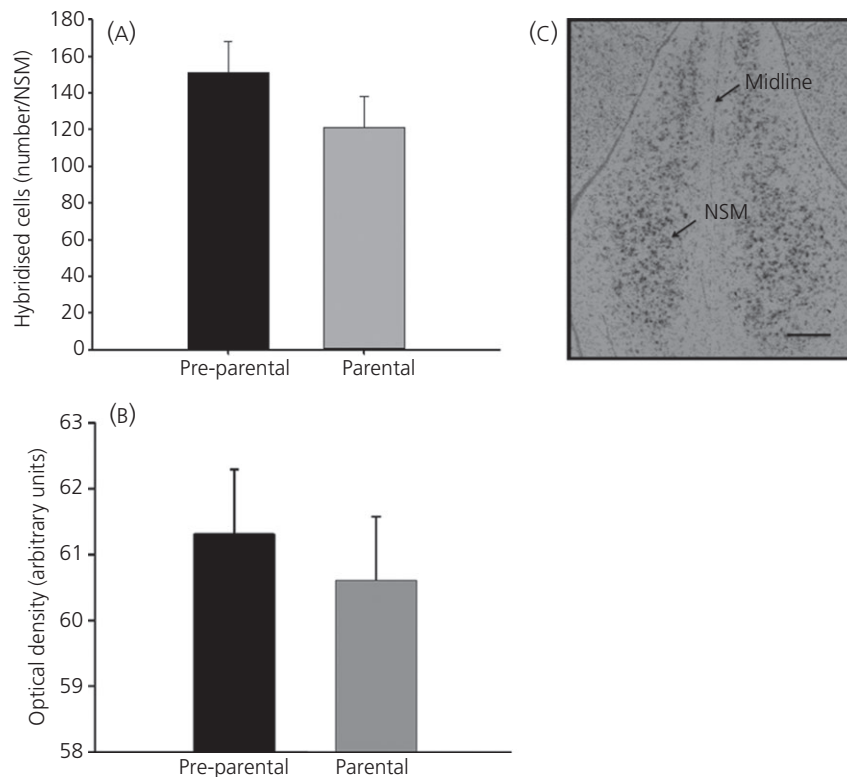


Fig. 6. There was no significant difference in (A) the number of cells hybridised or (B) the optical density for mineralocorticoid (MR) receptor mRNA in pre-parental ($n = 8$) and parental ($n = 8$) male Gambel's white-crowned sparrows in the nucleus septalis medialis. All values are presented as the mean \pm SEM. $P > 0.05$ Representative photomicrographs of autoradiography in bright field showing cells in (C) the nucleus septalis medialis. NSM, nucleus septalis medialis. Scale bars = 200 μ m.

A shift in the MR : GR ratio may be one potential mechanism for regulating the stress response (45,52). Interestingly, in a study performed on rats comparing either over-expression or under-expression of GR and MR, a combination of high MR expression with low GR expression resulted in enhanced negative-feedback compared to low GR and normal MR (52). Whether the ratio of MR to GR, in terms of feedback, occurs in the same pattern in mammals as in birds remains to be determined, although some evidence exists. In House sparrows (*Passer domesticus*), seasonal variation in HPA axis activity occurs, with higher stress-induced levels occurring with a reduced MR : GR ratio (53). This same pattern is observed in House sparrows, at the forefront of their range expansion, and also in Zebra finches, selected for a high stress response; both examples have increased HPA axis activity with a reduced MR : GR ratio, as well as a trend towards lower MR expression (37,54). Conversely, evidence from chronically stressed mammals and birds indicated that reductions in MR or GR can lead to reductions in the stress response (27,55–57). This line of evidence would suggest that decreases in MR expression, and thus disruption of the ratio, could lead to altered HPA axis activity in Gambel's white-crowned sparrows. It is unlikely that the change in the ratio of MR : GR in Gambel's white-crowned sparrows breeding on the North Slope of Arctic Alaska is a result of chronic stress because food resources were plentiful, snow had melted, predation pressure was low and

baseline stress values were similar to those analysed during the winter months (58). In addition, there was no change in fat levels, although there was a significant increase in mass. The increase in mass during the parental stage may be attributed to behavioural changes such as singing and mate guarding, which allows for increased foraging, or the anabolic effects of androgens (35,59). It should be noted that this is not a consequence of chronic stress. Thus, changing the MR : GR ratio could be an adaptive mechanism for altering HPA axis activity.

Conclusions

The present study is the first to describe stress hormone receptor changes in the regulation of the stress response in free-living Arctic male birds. MR distribution was widespread in the brain, whereas GR expression was restricted with limited expression in the hippocampus. Neurones expressing GR have been previously reported to impinge upon MR neurones in the hippocampus and to participate in the regulation of circulating levels of corticosterone. As a result of the low levels of expression in the hippocampus, GR feedback may be more important in other brain regions, including the PVN and pituitary gland. MR expression was reduced as males transitioned from the pre-parental to the parental stage, which is associated with a decrease in the stress response. This reduction in MR expression

could be mediating changes in HPA axis sensitivity by adjusting the ratio of MR : GR. In addition, we do not know whether there are changes in the distribution of MR in the cellular compartment or in the membrane, which may influence the way in which the MR responds to stress. Changes in MR may be more critical in regulating behavioural responses to stressors or acting as a neural protective mechanism in addition to controlling negative-feedback. We cannot disregard the possibility that regulation could be occurring at other levels of the brain such as the GABAergic neuronal network or simply at the cognitive level for interpreting the severity of the situation. The reduction in MR expression could be associated with a decrease in behavioural sensitivity to stress to promote parental investment and stave off nest abandonment, which is important in the brief breeding season in the Arctic

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